(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 June 2001 (07.06.2001)

PCT

(10) International Publication Number
WO 01/40801 A2

(51) International Patent Classification[7]: G01N 33/569

(21) International Application Number: PCT/US00/32749

(22) International Filing Date: 1 December 2000 (01.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/168,926 3 December 1999 (03.12.1999) US

(71) Applicant: MERIDIAN DIAGNOSTICS, INC. [US/US]; 3471 River Hills Drive, Cincinnati, OH 45244 (US).

(72) Inventor: WILLIS, David, H., Jr.; 1562 Muskegon Drive, Cincinnati, OH 45255 (US).

(74) Agents: LEVY, Mark, P. et al.; Thompson Hine & Flory LLP, 2000 Courthouse Plaza N.E., P.O. Box 8801, Dayton, OH 45401-8801 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/40801 A2

(54) Title: DIAGNOSIS AND TREATMENT FOR HELICOBACTER PYLORI INDUCED COLIC

(57) Abstract: The present invention provides a method for determining the cause of colic in infants and providing treatment therefor. In accordance with the method of the present invention, an infant exhibiting symptoms of colic is tested to determine if the symptoms could be attributable to an *H.pylori* induced infection and, if the test is positive, the infant is treated for the infection using an accepted treatment method.

DIAGNOSIS AND TREATMENT FOR HELICOBACTER PYLORI INDUCED COLIC

Cross-Reference to Related Applications

This application claims priority from provisional U.S. patent application Serial No. 60/168,926 filed December 3, 1999.

Background

Colic in young children normally can not be attributed to any particular cause. There are numerous "old wife's tales" regarding the cause of this condition, teething being one of the most commonly suspected causes. The lack of a reliable diagnosis for this condition is frustrating to the pediatrician and the parent alike.

The present invention relates to the applicant's discovery that the Helicobacter Pylori (*H. pylori*) bacterium has been implicated in a significant percentage of infants suffering from colic. Briefly, *H. Pylori* is a bacterium that is generally found in the upper gastrointestinal tract of humans and has previously been implicated in gastroduodenal diseases such as peptic ulcers, gastritis and other maladies. The bacterium was originally classified as a Campylobacter and then reclassified as a Helicobacter based on more detailed information regarding its ultrastructure and fatty acid composition. Recent studies have found a relatively high percentage of the *H. Pylori* bacterium in infants suffering from colic of unknown origins. In fact, these same studies have indicated that incidence of colic actually caused by *H. pylori* infection could be as high as 45% in symptomatic patients! Specifically, the results of these studies have shown that the incidence of *H. pylori* in neonatal (<3 months old) patients was five to six times higher (43% vs 7%) in colic patients than in age and geographically matched controls. There is also anecdotal information that some of the *H. pylori* positive children who were given H2 blockers to alleviate symptoms, had their *H. pylori* infections clear and colic symptoms abate. It is the results of these studies, applicant's observations of the same, and applicant's experimentation in this area that form the basis for the present patent application.

As mentioned above, these recent studies indicate that as many as 45% of colic of unknown origins in infants may be attributable to the *H. pylori* bacterium. Needless to say,

given the high incidence of colic of unknown origin in the infant/neonatal population and the resultant negative effects on the health of infants suffering therefrom, effective and efficient methods to diagnose and treat infants inflicted with colic would be highly desirable. In fact, even if only a relatively small percentage of the currently undiagnosed and untreated colic cases were appropriately dealt with through applicant's invention, the benefits to infants, and their parents would be immeasurable.

Summary of the Invention

The present invention provides a heretofore unknown method for diagnosing and treating previously undiagnosed colic (i.e. colic of unknown origins) in infants. More specifically, due to the recent implication of the *H. pylori* bacterium as the cause of many previously undiagnosed incidences of colic mentioned above, the present invention provides that infants suffering from previously undiagnosed colic be tested for *H. pylori* infection using an effective method for determining the presence of the *H. pylori* bacterium. The invention also provides that infants testing positive for the *H. pylori* induced infection be treated using an approved therapy for clearing up the infection. While the present invention specifically provides many known methods for diagnosing and treating the *H. pylori* bacterium induced infections that would be suitable and operable in accordance with the method disclosed herein, it is to be understood that the applicant's invention is not intended to be limited solely to these illustrative examples of preferred embodiments. Furthermore, other objects and advantages of the present invention will be apparent from the following description and the appended claims.

Detailed Description

In accordance with the method of the present invention, an infant exhibiting previously undiagnosed symptoms of colic is subjected to testing to determine if the symptoms could be attributable to an *H. pylori* induced infection. Any of the numerous known methods for detecting *H. pylori* infection may be used in accordance with the invention, many of which are referenced in the literature. These include the urea breath test, gastric biopsy, fecal culture, fecal PCR, saliva culture and blood serology for *H. pylori* antibodies. Examples of

the latter techniques are found in U.S. Patent 5,262,156 to Aleonohammad and European Patent Application 0 329 570 to Blaser. Other known methods for detecting *H. pylori* infection that may be used in accordance with the present invention include the serum antibody test, the urine antigen or antibody test, the urea blood test, endoscopy, rapid urease of an endoscopic biopsy, or PCR, among others.

In a preferred embodiment an assay which is simple to use, relatively inexpensive, and non-invasive would be highly desirable. In accordance therewith, a recently developed fecal immunoassay available from Meridian Diagnostics as described in US Patent 5,716,791, the contents of which are herein incorporated by reference, is considered to be useful for practicing the method of the present invention. The non-invasive nature of this assay makes it particularly desirable for use in detecting *H. pylori* infection in infants. In brief, that assay method comprises:

dispersing a fecal specimen suspected of carrying *H. pylori* in a sample diluent;

contacting the fecal specimen in the diluent with a first polyclonal antibody for *H. pylori* antigen to form a complex of the antibody and the antigen;

separating said specimen from said complex;

exposing the complex to a second polyclonal antibody for said antigen and a portion of the antibody reacting with said complex, one of said first and second antibody being bound to a solid carrier and the other being labeled with a detection agent; and

detecting the labeled antibody and in turn determining the presence of *H. pylori* antigen in said fecal specimen. This method can be varied by employing monoclonal antibody which is genus specific for Helicobacter or Campylobacter in place of one of the polyclonal antibodies. Such genus specific antibodies are commercially available. A mixture of monoclonal *H. pylori* specific antibodies can be used in place of the polyclonal.

The immunoassay can be embodied in a kit including a plate of antibody-coated wells, sample diluent, the labeled antibody, e.g., an enzyme-antibody conjugate, wash buffer and, in the case of an ELISA, a substrate solution.

Whatever method is used to determine the presence of the *H. pylori* bacterium in an infant exhibiting symptoms of colic, whether one of the methods specifically delineated herein or another acceptable method, in those cases in which *H. pylori* infection is detected, the infant is then treated using an accepted treatment regimen in accordance with present invention. Though numerous antibiotics have activity against *H. pylori* in vitro, therapy with a single antibiotic is generally ineffective in clinical practice. This is due to several factors including the acid environment of the stomach which can decrease the effectiveness of certain antibiotics and the protection afforded by the gastric mucous gel which *H. pylori* inhabits. Successful therapies for *H. pylori* usually involve 2 to 4 drugs given for periods of 7-14 days. Nonetheless, the applicant has observed that a treatment regimen with an H2-blocker may be sufficient to eradicate *H. pylori* infections in some colic patients.

Examples of acceptable treatment therapies for curing a diagnosed *H. pylori* infection in infants exhibiting colic symptoms include the original therapy (the so-called bismuth triple therapy) which consists of the administration of tetracycline, bismuth, and metronidazole for a period of 14 days. This therapy has been approved for use by the United States Food and Drug Administration (FDA). In US trials, the therapy has been found to have an eradication rate between 77-82%. A 7 day course of traditional bismuth triple therapy may also be effective but has not been adequately validated in large US trials. Adding a proton pump inhibitor (PPI) appears to enhance the efficacy of bismuth triple therapy (so called quadruple therapy). Using amoxicillin in place of tetracycline results in lower rates of *H. pylori* eradication. Side effects occur in 30-50% of patients though fewer than 5% have to discontinue therapy.

Another acceptable method of treatment in accordance with the present invention would include the use of two drugs, namely clarithromycin and either a PPI or ranitidine bismuth citrate (RBC). This method was among the first regimen for treating *H. pylori* to achieve widespread use and has yielded eradication rates in the range of 64-84%. Other acceptable methods for treatment would include the following three dual therapies (omeprazole and clarithromycin; lansoprazole and clarithromycin; ranitidine bismuth citrate and clarithromycin) that have all previously been approved by the FDA for use within the US. Details with respect to dosage and duration for these therapies can be found in Table 1 below.

In general, these dual therapies have exhibited better tolerance than the bismuth triple therapy noted above. Side effects often associated with the use of clarithromycin include altered taste, nausea, diarrhea, and headache. Despite being simple and well tolerated, the current dual regimens have all but been replaced in clinical practice by triple drug therapies which offer superior efficacy.

It has also been found that liquid Zantac has been effective in clearing *H. pylori* infections in clinical trials. This information would appear to suggest that H2 blockers as a class may be effective in eradicating *H. pylori* infections. Accordingly, it is believed that other H2 blockers not specifically disclosed herein may exhibit efficacy in the treatment of *H. pylori* induced colic in infants. As such, the use of these H2 blockers to treat previously undiagnosed incidences of colic in infants is considered within the scope of the present invention.

PPI or RBC-based triple therapies (see Table 1) are effective in eradicating *H. pylori* infection and well tolerated by patients. These regimens consist of a PPI or RBC in combination with 2 antibiotics (some combination of amoxicillin, clarithromycin, or metronidazole). Medications are given twice daily for periods of 7 to 14 days. Particularly notable are the combinations of a PPI or RBC, clarithromycin, and either amoxicillin or metronidazole which have consistently achieved eradication rates of $>85\%$. The combination of lansoprazole, clarithromycin and amoxicillin given twice daily for 14 days has recently achieved FDA approval in the US.

Recently, several other antimicrobials have been used to treat *H. pylori* with varying degrees of success. Furazolidone is a synthetic nitrofuran antibiotic with a broad spectrum of action. Furazolidone (100 mg qid) has been used in place of metronidazole in a number of treatment regimens for *H. pylori* and appears to be effective even for many *H. pylori* strains resistant to metronidazole. A disadvantage of furazolidone is its expense within the US. Nonetheless, as the prevalence of metronidazole and macrolide resistant *H. pylori* strains increases, alternative medications such as furazolidone will take on greater importance.

Other macrolides such as azithromycin exhibit excellent activity against *H. pylori* in vitro but give poor results in vivo. As an example, azithromycin has a long biological

half-life, leading some to speculate that it could be given for periods as short as 3 days. Unfortunately, with a few exceptions, the studies using azithromycin have yielded disappointing results. It appears that azithromycin may be effective when given in high doses (1 gram) for at least 7 days but this remains to be proven in large studies. At present, azithromycin and other mactdides should be regarded as interesting but unproven antimicrobial agents for *H. pylori*.

The quinolone antibiotic, ciprofloxacin, has also been used to treat *H. pylori* in several small studies. For now, ciprofloxacin like azithromycin should not be considered in the routine treatment of *H. pylori* infection. Rather, these agents should only be considered in those patients allergic to more established therapies or in the setting of treatment failure. Agents such as furazolidone and perhaps ciprofloxacin may prove useful particularly when resistance to metronidazole and/or clarithromycin are strongly suspected or proven with specialized testing. Duration of therapy and antibiotic dosing appear to influence the effectiveness of therapy. In many parts of Europe, the accepted duration of triple therapy for *H. pylori* infection is 7 days. Numerous large European trials have reported an eradication rate with 1 week triple therapy of >85%. The report of the recent Digestive Health Initiative International Update Conference on Helicobacter pylori recently recommended that therapy be given for 14 days in the US. Several 10 day treatment programs are probably as good as their 14 day counterparts. The first 10 day triple therapies should achieve FDA approval in 1998. Representative examples of many of the antibiotic therapies discussed herein are summarized in the following Table 1.

Table 1

Approved Therapies for Treatment of *H. pylori* Induced Infections

Generic Name	Adult Dosing	Duration
Dual therapies		
omeprazole	20 mg BID	28 days
and		
clarithromycin	500 mg TID	14 days

ranitidine bismuth citrate and	400 mg BID	28 days
clarithromycin	500 mg TID	14 days
lansoprazole and	30 mg TID	14 days
amoxycillin	1,000 mg TID	14 days
"Triple" therapies		
bismuth subsalicylate	Two tablets (525 mg) QID	14 days
or		(*see note below)
bismuth subcitrate	One tablet (120 mg) QID	14 days
and		
metronidazole and	250 mg QID	14 days
tetracycline and	500 mg QID	14 days
H2 antagonist	dose to heal ulcer	28 days
lansoprazole and	30 mg BID	14 days (*see note below)
amoxycillin and	1,000 mg BID	14 days
clarithromycin	500 mg BID	14 days
Quad therapies		
bismuth subsalicylate	Two tablets (525 mg) QID	7 days
or		(*see note below)

bismuth subcitrate	One tablet (120 mg) QID	14 days
and		
metronidazole	250 mg QID	7 days
and		
tetracycline	500 mg QID	7 days
and		
omeprazole	20 mg BID	7 days
or		
lansoprazole	30 mg BID	7 days
omeprazole	20 mg BID	10-14 days
or		(*see note below)
lansoprazole	30 mg BID	10-14 days
and		
clarithromycin	500 mg BID	10-14 days
and		
metronidazole	500 mg BID	10-14 days
omeprazole	20 mg BID	10-14 days
or		
lansoprazole	30 mg BID	10-14 days
and		
clarithromycin	500 mg BID	10-14 days
and		
amoxycillin	1,000 mg BID	10-14 days
rantidine bismuth	400 mg BID	10-14 days
citrate and		
clarithromycin	500 mg BID	10-14 days
and		
amoxycillin	1,000 mg BID	10-14 days

**Note: In some countries they have found that 7 days therapy with this regimen is equally effective.*

All of the drugs listed above are prescribed as fixed doses, with no accounting for patient age or size. This stems from the fact that these protocols have been approved for use in adults only. In accordance with the present inventions, the dosages will be adjusted for treatment of infants in manners well known in the art. Specifically in this regard it is noted that there are current ongoing studies being conducted by several drug companies to achieve eradication of *H. pylori* in pediatric patients. These studies involve drug combinations which have altered the established dosing for adults for administration to infants by changing the doses to an age or weight basis, with additional changes to the number and frequency of drug administration as necessary. Thus, expected changes can be generally classified as from fixed dose to patient age adjusted dosing, patient weight adjusted dosing, patient surface area adjusted dosing, period of dosing, size/frequency of doses, multi-drug pills to reduce number of pills to be administered.

Having described the invention in detail and by reference to specific embodiments thereof it will be apparent that there numerous variations and modifications are possible without departing from the spirit and scope of the following claims.

What is claimed is:

1. A method comprising the steps of:

testing an infant exhibiting colic for *H. pylori* infection, and.

treating said infant for *H. pylori* infection if said testing is positive for *H. pylori* infection.

2. The method of claim 1 wherein said infant is tested for *H. pylori* infection using a fecal assay.

3. The method of claim 2 wherein said fecal assay is a sandwich assay.

4. The method of claim 3 wherein said fecal assay employs a first antibody which is labeled and a second antibody which is carried on a support.

5. The method of claim 1 wherein said infant is tested using a serum assay.

6. The method of claim 5 wherein said serum assay employs *H. pylori* antigen or a mixture of *H. pylori* antigens.

7. The method of claim 1 wherein said step of treating said infant for *H. pylori* infection comprises administering to said infant an antibiotic treatment regimen.

8. A method which comprises testing an infant suffering from colic for *H. pylori* infection.

9. The method of claim 1 wherein said infant is tested using a urea breath test.

10. The method of claim 1 wherein said infant is tested using a urea blood test.

11. The method of claim 1 wherein said infant is tested using endoscopy.

12. The method of claim 1 wherein said infant is tested using rapid urease of an endoscopic biopsy.

13. The method of claim 1 wherein said infant is tested using a serum assay.

14. The method of claim 1 wherein said infant is tested using a urine antigen or antibody test.

15. The method of claim 1 wherein said infant is tested using PCR.

16. The method of claim 1 wherein said step of treating said infant for *H. pylori* infection comprises administering to said infant a treatment regimen comprising H2 blockers or Proton-Pump Inhibitors.

17. The method of claim 1 wherein said step of treating said infant for *H. pylori* infection comprises administering to said infant a treatment regimen comprising liquid Zantac.

18. A method for diagnosing and treating colic in infants comprising the steps of:

(a) collecting a smear of a fecal specimen from an infant exhibiting colic symptoms on a substrate;

(b) immersing at least the portion of the substrate carrying the smear in a sample diluent so as to disperse the fecal specimen in the diluent;

(c) contacting the fecal specimen in the diluent with a first polyclonal antibody for *H. pylori* antigen to form a complex of the antibody and the antigen;

(d) separating said specimen and said complex;

(e) exposing the complex to a second polyclonal antibody for said antigen and a portion of the antibody reacting with said complex, one of said first and second antibody being bound to a solid carrier and the other being labeled with a detection agent;

(f) determining the amount of the labeled antibody and in turn determining the presence of *H. pylori* antigen in said fecal specimen;

(g) upon finding the presence of *H. pylori* in said fecal sample, administering a treatment regimen to said infant for eradicating an *H. pylori* induced infection.

19. The method of claim 18 wherein said treatment regimen is selected from the group consisting of antibiotics, H2 blockers and proton-pump inhibitors.

20. The method of claim 18 wherein said treatment regimen is selected from the group consisting of the bismuth triple therapy, antimicrobials, the bismuth triple therapy plus PPI, dual therapy antibiotics, and liquid Zantac.